UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM **June 13, 2019**

SUBJECT: Preliminary Technical Screen of the Section G Presented for the New Product OX5034 (EPA File Symbol: 93167-EUP-E) Containing the Tetracycline-Repressible Transactivator Protein Variant (tTAV-OX5034; Designated as the New Active Ingredient) Protein, a Variant of the Modified *Discosoma* spp. DsRed2 Protein (Designated as a New Inert Ingredient), and the Genetic Material (Vector pOX5034) Necessary for Their Production in OX5034 *Aedes aegypti*. Data and Information Were Provided in Support of a FIFRA Section 5 Application.

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FROM: Amanda A. Pierce, Ph.D., Biologist
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

AMANDA PIERCE Digitally signed by AMANDA PIERCE Date: 2019.06.13 14:06:31 -04'00'

TO: Eric W. Bohnenblust, Ph.D., Risk Manager
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

THROUGH: Eric W. Bohnenblust, Ph.D., Risk Manager
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

Mike Mendelsohn, Branch Chief
Emerging Technologies Branch
Biopesticides and Pollution Prevention Division (7511P)

I. BACKGROUND

Oxitec Ltd., (Oxitec or the applicant) requests an Experimental Use Permit (EUP) under FIFRA section 5 for a new end-use product containing the new active ingredient tetracycline-repressible transactivator protein variant (tTAV-OX5034) protein, the new inert ingredient DsRed2-OX5034 protein, and the genetic material (vector pOX5034) necessary for their production in OX5034 *Aedes aegypti* (Yellow Fever mosquito). Oxitec requests this EUP to evaluate whether the product is efficacious in suppressing naturally-occuring *Ae. aegypti* populations under field conditions.

OX5034 is described as a species-specific female larvicide, or "male-selecting" larvicide, that results in all-male progeny in the absence of tetracycline in the larval diet. With continued field releases of OX5034 homozygous males, the *Ae. aegypti* population is expected to progressively decline due to the reduced number of females emerging in the area. The same male-selecting trait is also harnessed in the manufacture of OX5034 to remove transgenic females from the mosquito population prior to the field releases, ensuring female mosquitoes are not released.

The applicant requests a 24-month EUP for a cumulative annual test area of 5,000 acres. The area is divided into multiple test and control plots within Monroe, Co., Florida and Harris, Co., Texas. Under the EUP, Oxitec is planning to test the efficacy of the product by deploying eggs, pupae, and adults homozygous for the OX5034 trait.

In support of this application, Oxitec submitted a protocol (section G) detailing the objectives of the proposed testing and a detailed description of the proposed testing program.

II. SUMMARY

The results of the preliminary technical screen of these materials are summarized below.

Overarching deficiencies:

- Each release mechanism/life stage (egg, pupae, adults) must be tested separately and must have label directions corresponding to its use. It is unclear how many replicates will be tested for each release mechanism and for each trial type. The application also mentioned testing of different application rates. However, it is again unclear how many replicates per application rate will be tested and which release mechanisms will be used. The lowest labeled release rate for each release mechanism must be replicated a minimum of three times. Given the large number of variables, it is unclear if the requested 5,000 acres is sufficient. The number of treatments, replicates, and necessary acres must be clarified. Update Table 1 to reflect these clarifications.
- As referenced in EPA's letter dated 2/14/19, efficacy is determined based on effectiveness of the product not "efficacy of the active ingredient". Therefore, comparisons for efficacy determination need to be made between treated vs. untreated areas. The Section G must be updated accordingly.
- For efficacy analysis, use generalized linear mixed models for binomially distributed outcomes to analyze the data rather than ANOVAs using an arcsine transformation.

Specific deficiencies and shortcomings:

- Label
  - Egg, pupae, and adult releases require their own label directions, and each must be tested separately according to the appropriate label directions.
  - The lowest release rate for each label must be tested. This is also true for the longest interval between mosquito box/pot replacements.
  - Clarify in the Section G why acreage coverage differs for egg/pupal releases vs. adult releases.
  - Page 13 of the Section G states "mosquito rearing boxes, plastic pots, or bug dorm cages" will be used for adult releases. The label specifies "release pots". This discrepancy must be clarified.
- Experimental design
  - Page 5 of the Section G states that Trial A and Trial B will be completed using a "phased approach". This makes sense given that the results from Trial A appear to influence the design of Trial B. However, page 8 states "Trial A and Trial B may take place simultaneously". Clarify the order in which trials take place.
  - Provide a general timetable for releases. Specifically, the projected length of time for Trial A and Trial B along with any overlap and the projected length of time for post-release monitoring. Include any relevant information regarding mosquito biology (e.g., length of season, time of diapause, etc.).
  - Clarification is needed regarding the trapping interval for Trial A and Trial B as well as justification for the density of traps. For example, when will trapping begin in relation to initial and subsequent releases? Will the high density of traps near the release pots reduce efficacy?
  - Page 15 of the Section G states that an objective of Trial B is to detect the presence of an observable effect in natural breeding sites. Additional information is needed on how a "natural breeding site" is defined, how will the sites be identified, how will an effect be measured, and whether measurements will be considered part of efficacy calculations.
  - Remove the statement that "indoor placement of ovitraps may be used".
  - OX5034 males will be dusted prior to release to distinguish between released males and males from subsequent generations. How will this be done for males released from egg/pupal boxes?
  - Per EPA's letter dated 2/14/19, post-release monitoring must continue through at least two generations without any observed fluorescence.
  - Persistence monitoring must include qPCR testing as described in the mortality assessment.
  - The number of traps must be increased during persistence monitoring.
  - For persistence monitoring in Trial B, monitoring must be expanded beyond the treatment area.

- Statistical analysis
    - Use generalized linear mixed models (GLMM) for binomial distributions to analyze the efficacy data. GLMM can account for the correlation between the replicates within each site. Include site as a random effect in the model. The data (number surviving females/N total females) of each replicate can be directly analyzed by GLMM without any transformation. Log link or logit link function can be used in the models. The treated:control (T/C) ratio and its 95% CI can be estimated from the GLMM. Then, the ratio T/C (and 95% CI) estimated from GLMM can be used to calculate the efficacy (and 95% CI) as follows:

      $$E = 100\times(C-T)/C = 100\times(C/C - T/C) = 100\times(1 - T/C)$$

    - When evaluating dispersal difference of released OX5034 mosquitoes, mean and max values are appropriate/meaningful statistics for normal distributions. However, if data are not normally distributed, median and max values are better for characterizing the distributions. Report the following descriptive statistics (e.g., p25, p50, mean or median, p75, p90, and max).
    - For persistence monitoring, duration (time until disappearance of fluorescent larva) is time to event. The data are all completed data since the duration at each site is followed until no individuals have been found. Kaplan-Meier Estimator (median and 95% CI) and descriptive statistics (e.g., min, p25, median, mean, p75, p90, and max) should be used to characterize the distributions.